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DATE: **February 3, 2000**

SUBJECT: ***DISULFOTON:*** **REVISED (**2nd) Report of the Hazard Identification Assessment Review Committee.

FROM: David G. Anderson, Toxicologist
Reregistration Branch-2
Health Effects Division (7509C)

THRU: Jess Rowland, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Alan Nielsen, Branch Senior Scientist
Reregistration Branch-2
Health Effects Division (7509C)

PC Code: 032501

On January 19, 2000, the Health Effects Division's Hazard Identification Assessment Review Committee evaluated the toxicology data base of disulfoton, re-assessed the evidence for neurotoxicity induced by disulfoton exposure. The Committee also addressed the potential sensitivity of infants and children as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

Members in Attendance

Hazard Identification Assessment Review Committee members in attendance: William Burnum, Jess Rowland, Pamela Hurley, David Nixon, Elizabeth Mendez, Nicole Paquette, and Brenda Tarplee (Executive Secretary).

Members in absentia Beth Doyle, Vicki Dellarco, and Tina Levine.

Others in attendance were Pauline Wagner and Christina Jarvis.

Data Presentation:
and
Report Preparation

David G Anderson
Toxicologist

I. INTRODUCTION

On April 25, 1996.the Health Effect's Division RfD/Peer Review Committee evaluated the toxicology data base of Disulfoton and established the Reference Dose (RfD) of 0.0003 mg/kg/day based on a NOAEL of 0.025mg/kg/day and an Uncertainty Factor of 100 for inter species extrapolation and intraspecies variation *(Memorandum*: G.Ghali to G. LaRoca, April 21, 1997).

On May 14, 1996.the Toxicology Endpoint Selection Committee selected the doses and endpoints for acute dietary and occupational as well as residential exposure risk assessments (TES Document 6/5/96).

On November 20, 1997, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) re-evaluated the toxicology data base, re-assessed the RfD and selected the toxicology endpoints for acute dietary as well as occupational and residential exposure risk assessments. In addition, the HIARC also addressed the potential enhanced susceptibility of infants and children from exposure to disulfoton as required by the Food Quality Protection Act (FQPA) of 1996.

On April 9, 1998, the HIARC reviewed the results of a two-generation reproduction study in rats (MRID# 44440801) that was recently submitted to the Agency and the impact of this study in the doses and endpoints selected for the various risk assessments.

On May 12-14, 1998, the HIARC conducted a comprehensive review of 40 organophosphates, including disulfoton. At this meeting it was concluded that the toxicology database is inadequate since there was a data gap for an acceptable acute delayed neurotoxicity study in the hen. Subsequently, the requirement of a developmental neurotoxicity study was **'reserved'** at this time.

On January 19, 2000, the HIARC reviewed the results of a new acute delayed neurotoxicity study in the hen. In addition, the equivocal results of a 90-day neurotoxicity study in rats were reviewed for potential disulfoton induced neuropathy. HIARC also evaluated the toxicology data base for disulfoton to determine whether a DNT was triggered. **None of the endpoints for any of the RfDs or occupational or residential exposure were changed from the previous HIARC**. The final committee determination are presented below.

II. HAZARD IDENTIFICATION

A. Dietary Hazard

1. Acute Reference Dose (Acute RfD)

Study Selected: Acute Neurotoxicity - Rat §81-8

MRID No. 42755801

Executive Summary: In an acute neurotoxicity screening study, disulfoton (97.8% pure) was administered in a single gavage dose to 10 male Sprague-Dawley rats at doses of 0,

0.25, 1.5, or 5.0 mg/kg and to 10 female Sprague-Dawley rats at doses of 0, 0.25, 0.75 or 1.5 mg/kg. These rats were assessed for reactions in functional observational battery (FOB) and motor activity measurements at approximately 90 minutes post-dosing and on days 7 and 14. Cholinesterase determinations (erythrocyte and plasma) were made at 24 hours post-dosing. Six rats/sex/dose were examined for neuropathological lesions.

At 0.75 mg/kg, 4/10 females had muscle fasciculations. At 1.5 mg/kg, males had muscle fasciculations, diarrhea, and sluggishness and females also had tremors, ataxia, oral staining, decreased activity/sluggishness, decreases in motor and locomotor activity (38–49% of control), and a slightly increased duration of nasal staining. One female at 1.5 mg/kg died from cholinergic intoxication on the day of dosing. At 5.0 mg/kg, males also had symptoms similar to those observed in females at 1.5 mg/kg/day, including reduced motor/locomotor activity (36–45% of control). Recovery appeared to be complete in surviving animals by Day 14. Based on the evidence of neurotoxicity (probably associated with inhibition of cholinesterase) in females at 0.75 mg/kg, the study LOAEL is 0.75 mg/kg and the study NOAEL is 0.25 mg/kg.

At 0.75 mg/kg in females, cholinesterase activities were inhibited by 53% (erythrocyte) and 30% (plasma) and by 75% (erythrocyte) and 52% (plasma) at 1.5 mg/kg in females. At 5.0 mg/kg in males, cholinesterase activities were inhibited by 21% (erythrocyte) and 25% (plasma). The LOAEL for inhibition of cholinesterase activity is 0.75 mg/kg and the NOAEL for inhibition of cholinesterase activity is 0.25 mg/kg.

Dose and Endpoints for Risk Assessment: NOAEL= 0.25 mg/kg based on neurotoxicity signs, plasma and erythrocyte cholinesterase inhibition in female rats.

Comments about the study and/or Endpoint: This dose and endpoint is appropriate since the toxicological effects were observed following a single oral dose.

Uncertainty Factors (UF): 100 (10 x for inter-species extrapolation, 10 x for intra-species variability.

$$\textbf{Acute RfD} = \frac{\textbf{0.25 mg/kg (NOAEL)}}{\textbf{100 (UF)}} = \textbf{0.0025 mg/kg}$$

This risk assessment is required.

2. Chronic RfD

Study Selected: Chronic Feeding Dog §83-1

MRID No. 44248002

Executive Summary: In a chronic toxicity study, disulfoton (97% a.i.%) was administered orally in the diet to purebred beagle dogs (4/sex/dose) at dose levels of 0.5, 4 or 12 ppm (equivalent to 0.015, 0.121 and 0.321 mg/kg/day for males; and 0.013, 0.094 and 0.283 mg/kg/day for females) for one year. Potential ocular and neurologic effects were addressed. Plasma cholinesterase was decreased starting at day 7 in the 4.0 ppm dose groups of the study through to termination (males 39% to 46%; females 32% to 45%). Erythrocyte cholinesterase was decreased starting at day 91 in the 4.0 ppm dose groups through to termination (males 23% to 48%; females 17% to 49%). Not all the values at 4.0 ppm were statistically significant, probably because of the wide range in values, but at least 2 animals per group showed biologically significant cholinesterase inhibition. By termination cholinergic effects of the plasma, erythrocytes, brain, and ocular tissues were observed in both sexes in the 4 and 12 ppm treatment groups. Plasma and erythrocyte cholinesterase depression are compared to pretreatment values. Brain, cornea, retina and ciliary body cholinesterase depression are compared with concurrent control values at termination only. In the 12 ppm treatment groups, depressed cholinesterase was observed in plasma (56%-63%), erythrocytes (30%-91%), and brain (32%-33%) compared to their respective controls. In the 4 ppm treatment groups in males and females, cholinesterase was depressed in plasma (38%-46%), erythrocytes (40%- 38%), and brain (females only, 22%). Disulfoton inhibited cholinesterase of the cornea, retina, and ciliary body, but did not appear to alter the physiologic function of the visual system. In the 12 ppm treatment groups, depressed cholinesterase was observed in the cornea (60-67%), ciliary body (45-54%), and retina (males only; 67%). In the 4 ppm treatment groups, cholinesterase was inhibited in the cornea (50-60% lower), and retina (females only, 25%). No treatment-related ophthalmology findings or histological or electrophysiological changes in the retina were observed. No other treatment-related effects were observed. No animals died during the study. No treatment-related effects were observed in systemic toxicity including food consumption, body weights, clinical signs, hematology, clinical blood chemistry or urinalysis parameters, electrocardiogram, electroretinograms or clinical neurological findings, organ weights or gross or microscopic post-mortem changes in any treatment group. No neoplastic tissue was observed in dogs in the treatment and control groups. The LOAEL is 4 ppm (0.094 mg/kg/day), based on depressed plasma, erythrocyte, and corneal cholinesterase levels in both sexes, and depressed brain and retinal cholinesterase levels in females. The NOAEL is 0.5 ppm (0.013 mg/kg/day). These LOAEL/NOAEL for plasma cholinesterase inhibition extend from day 7 to termination and for erythrocyte cholinesterase inhibition they extend from day 91 to termination.

Dose and Endpoint for Establishing the RfD: The NOAEL is 0.5 ppm (0.013 mg/kg/day) based on depressed plasma, erythrocyte and corneal cholinesterase levels in both sexes and depressed brain and retinal cholinesterase levels in females.

Uncertainty Factors (UF): 100 (10 x for inter-species extrapolation, 10 x for intra-species variability.

Chronic RfD = $\frac{\text{0.013 mg/kg (NOAEL)}}{\text{100 UF}}$ = 0.00013 mg/kg

This risk assessment is required.

B. Occupational/Residential Exposure

1. Dermal Absorption; § 85-2

MRID No.: 43360201

Percentage absorbed: At 1, 4 or 10 hours, the following percentages of applied dermal doses were absorbed in the rat. Application site was washed after the 10 hour exposure and the 168 hour exposure (168 hour exposure data not given). Disulfoton is volatile and 10% to 30% of the applied dose was found to be volatile over a 10 hour period. The volatility of disulfoton is probably the reason for some of the low recoveries, but since volatility would also be present under field conditions it was not considered in the percentage absorption.

Dose in (μg/cm^2 on a 15 cm^2 site) & mg/kg based on 250 g rat	Exposure hours	Percentage absorbed
Concentration administered (0.85 μg/cm^2)		
0.051 mg/kg	1	5.9
0.051 mg/kg	4	13.9
0.051 mg/kg	10	26.0
Concentration administered (8.5 μg/cm^2)		
0.51 mg/kg	1	4.6
0.51 mg/kg	4	15.9
0.51 mg/kg	**10**	**36.2[a]**
Concentration administered (85 μg/cm^2)		
5.1 mg/kg	1	3.6
5.1 mg/kg	4	12.5
5.1 mg/kg	10	25.3

[a] = % dermal absorption factor chosen by TES of 5/14/96.

Dermal Absorption Factor: 36% at approximately 8.5 $\mu g/cm^2$ or 0.51 mg/kg for 10 hours should be used to convert oral studies to dermal studies where necessary.

Comments about the Study Endpoint: The TES Committee indicated that dermal absorption of 36%, obtained after 10 hours exposure at a concentration of 8.5 $\mu g/cm^2$ (0.51 mg/kg), should be used for correcting oral dosing to dermal dosing. If the exposure deviates by a large amount from 8.5 $\mu g/cm^2$ for 10 hours then a different percentage dermal absorption may be appropriate. The risk assessor should refer to the above table or HED Doc# 011316, MRID# 43360201, for a more complete understanding of the dermal absorption percentage and the relationship between percentage absorption and the dose applied to the skin. The HIARC concurred with the TES Committee on this approach for the use of the dermal absorption factor.

2. Short Term Dermal - (1-7 DAYS)

Study Selected: 21-day dermal study in rabbits §82-3

MRID No. 00162338

Executive Summary: In a 21-day dermal study, disulfoton, technical (97.8%) was administered dermally in a Cremophor EL emulsion to 5 New Zealand White rabbits/sex/group at 0, 0.4, 1.6 or 6.5 mg/kg/day for 15 separate exposures, 5 day/week for 6 hours/day for 21 days. No skin irritation occurred at any dose level. Females at the 6.5 mg/kg/day died (a total of 6) after 1-3 weeks of treatment and males (unknown numbers) died at 6.5 mg/kg/day after 3 days and 2 weeks of treatment. At 1.6 mg/kg/day, plasma cholinesterase was inhibited (41%) in females and (32%) in males after 1 week of treatment. At the same dose level, erythrocyte cholinesterase was inhibited (16% from pre-dosing values, but 21% from the concurrent control at 2 weeks and at termination 33% from control, but increased 3% from pre-dose values. Brain cholinesterase was marginally inhibited at 1.6 mg/kg/day in females (8%) and in males (7%) at termination (3-weeks).
The NOAEL was 0.4 mg/kg/day based on plasma, erythrocyte and brain cholinesterase inhibition in females and males.

Dose and Endpoint for use in risk assessment: NOAEL = 0.4 mg/kg/day was based on plasma, erythrocyte cholinesterase inhibition after 1 week of dosing.

Comments about study and/or endpoint: This endpoint and the NOAEL is supported by a developmental toxicity study in the rat. In that study the maternal NOAEL was 0.1 mg/kg/day based on 41% for both plasma and erythrocyte cholinesterase inhibition. When the 36% dermal absorption factor is used, the comparable dermal dose is 0.3 mg/kg/day [i.e., oral NOAEL of (0.1 mg/kg/day)/(0.36) = 0.3 mg/kg/day] The study represents cholinesterase inhibition after 2 weeks of dosing.

This risk assessment is required.

3. Intermediate Term O/R Exposure (1 Week to Several Months):

Study Selected - Special 6-months cholinesterase study.

MRID No.: 43058401

Executive Summary: In a 6-month study designed to establish a NOAEL and LOAEL for cholinesterase inhibition, technical grade disulfoton (98-99% pure) was administered in the diet to 35 male and female Fischer 344 rats for up to 6 months at levels of 0, 0.25, 0.5 or 1 ppm (approximate doses of 0, 0.02, 0.03 or 0.06 mg/kg/day for males and 0, 0.02, 0.03 or 0.07 mg/kg/day for females). At the end of 2, 4 and 6 months, 10 rats/sex/dose were taken for blood and brain cholinesterase assays. Statistically significant inhibition of cholinesterase activity was observed in erythrocytes in females at all doses (3-14% inhibition, 11-17% inhibition, and 23-29% inhibition at 0.24, 0.5, and 1.0 ppm, respectively. In addition, at 1.0 ppm, males had decreased erythrocyte cholinesterase activity (10-16% inhibition) and females had decreased plasma (8-17% inhibition) and brain (7-13% inhibition) cholinesterase activities. However, biologically significant and statistically significant inhibition of cholinesterase activity was observed only in the plasma, erythrocytes and brain of females at 1.0 ppm. No biologically significant inhibition of cholinesterase activity was observed in males. The LOAEL for inhibition of cholinesterase activity was 1.0 ppm is based on a 23-29% inhibition of erythrocyte, 12-17% inhibition of plasma and 13% inhibition of brain cholinesterase in females. The NOAEL is 0.5 ppm (0.03 mg/kg/day). No biological meaningful cholinesterase inhibition was observed in males at any dose level. Body weight, food consumption, and clinical signs were also monitored, but showed no treatment related effects. Based on these few parameters, no systemic effects were observed at any dose level and the NOAEL for systemic toxicity was 1.0 ppm (0.06 mg/kg/day for males and 0.07 mg/kg/day for females).

Dose and Endpoint for use in risk assessment: NOAEL=0.03 mg/kg/day was based on plasma, erythrocyte and brain cholinesterase inhibition in female rats.

Comments about study and/or endpoint: Since an oral NOAEL was identified, a dermal absorption factor of 36% should be used for this risk assessment. This endpoint is supported by similar effects (plasma, erythrocyte and brain cholinesterase inhibition) observed in a subchronic neurotoxicity study in rats (MRID# 42977401). In addition, the new 2-generation study on reproduction (MRID# 44440801) also supports the 6-month cholinesterase study endpoints.
The Committee considered a combination of factors in the decision to use the NOAEL of 0.5 ppm (0.03 mg/kg/day) from the 6-month cholinesterase study in rats for the this exposure assessment instead of the LOAEL of 0.5 ppm (0.03 mg/kg/day) from new 2-generation study on reproduction. Considered were that test material consumption was measured in the 6-month cholinesterase study and the measurements were invalid in the new 2-generation study on reproduction and the 6-month study was specifically designed to determine cholinesterase inhibition. Thus, mg/kg/day were measured in the 6-months study, but mg/kg/day dose levels in the reproduction study were approximated from

standard tables. In addition, adult P0 females showed marginal brain cholinesterase inhibition while the F1 adult females, dosed similarly, showed none.

This risk assessment is required.

4. Long-Term Dermal (Several Months to Life Time)

Study selected: Chronic Toxicity -Dog §83-1

MRID No. 44248002

Executive Summary: See summary under Chronic RfD.

Dose and Endpoint for Risk Assessment: NOAEL=0.013 mg/kg/day based on depressed plasma, erythrocyte and corneal cholinesterase levels in both sexes and depressed brain and retinal cholinesterase levels in females.

Comments about study and/or endpoint: This dose was used to establish the chronic RfD. Since an oral NOAEL was identified, a dermal absorption factor of 36% should be used for this risk assessment.

This risk assessment is required.

5. Inhalation Exposure (Any Time Period)

Study Selected: 90-Day Inhalation-Rat §82-4

MRID No.: 41224301

Executive Summary: Disulfoton was administered by inhalation to 12 Fisher 344 rats per sex per group for air control, polyethylene glycol-400: 50% ethanol vehicle control, 0.015, 0.15 or 1.5 mg/m^3 nominal dose levels for 90-days in a nose only chamber. The analytical determined mean dose levels were 0, 0, 0.018, 0.16 and 1.4 mg/m^3 for male and female rats. The rats were exposed to the test material 6 hours per day, 5 days per week. The particle sizes in the inhalation chambers had a MMAD ± geometric standard deviation of 1.3 ± 1.4, 1.1 ± 1.3, 1.0 ± 1.3 and 1.1 ± 1.4 μm for the two controls, 0.015, 0.15 and 1.5 mg/m^3 nominal dose levels, respectively. The range in mean daily particle sizes had a MMAD of 0.5 ± 1.0 μm to 2.6 ± 1.6 μm. At the highest dose level, plasma cholinesterase was depressed in males (19% and 14% from air controls at 38 days and term, respectively, $p \leq 0.05$) and in females (27% and 31% from air controls at 38 days and term, respectively, $p \leq 0.05$). Brain cholinesterase was depressed in males (29%) and females (28%) at termination. Erythrocyte cholinesterase was depressed in females at 38 days (11% at 38 days, $p \leq 0.05$, not considered biologically relevant) at 0.16 mg/m^3 and higher in males and females at 1.4 mg/m^3 at 38 days and term. Brain cholinesterase was depressed (10%, $p \leq 0.05$) at 0.16 mg/m^3, but this degree of variation was not considered biologically relevant due to variation noted in this parameter. Inflammation of the male nasal

turbinates occurred at 1.4 mg/m^3. No other test material related effects were noted. The NOAEL/LOAEL is 0.16 mg/m^3/1.4 mg/m^3 or 0.00016/0.0014 mg/L for plasma, erythrocyte and brain cholinesterase depression.

Dose and Endpoint for use in risk assessment: NOAEL=0.00016 mg/L based on plasma, erythrocyte and brain cholinesterase inhibition.

Comments about study and/or endpoint: This NOAEL will be used for inhalation exposure risk assessments for any time period (i.e., Short, Intermediate and Long-term). An inhalation toxicity study with 3 to 5 day exposure was available. In that study, the LOAEL was <0.0005 mg/L (lowest dose tested); a NOAEL was not established. Although this study could have been used for the Short-Term exposure risk assessment, the HIARC did not use this study because: (I) it demonstrated a LOAEL rather than a NOAEL; (ii) the use of a LOAEL would have required an additional 3 x UF; and (iii) the value derived from the use of the LOAEL and 3 UF (0.0005 ÷ 3= 0.00017 mg/kg/day) is comparable the NOAEL of 0.00016 mg/L in the 90-day study.

This risk assessment is required.

D. MARGINS OF EXPOSURE FOR OCCUPATIONAL/RESIDENTIAL) EXPOSURES

A Margin of Exposure (MOE) of 100 is adequate for occupational exposure risk assessments. The MOEs for residential exposure will be determine during risk characterization by the FQPA Safety Committee..

E. RECOMMENDATION FOR AGGREGATE EXPOSURE RISK ASSESSMENT

For aggregate exposure risk assessment , the MOE's derived for oral, dermal and inhalation exposures may be combined to obtain a total MOE since a common toxicological endpoint (cholinesterase) was observed during all routes of exposure (oral, dermal and inhalation) in the toxicity studies.

For Short-Term aggregate exposure risk assessment:

$$MOE_{total} = \frac{1}{\frac{1}{MOE_{(oral)}} + \frac{1}{MOE_{(dermal)}} + \frac{1}{MOE_{(inhalation)}}}$$

For Intermediate and Long-Term aggregate exposure risk assessment:

$$MOE_{total} = \frac{1}{\frac{1}{MOE_{(oral+\ oral\ dermal\ equivalent)}} + \frac{1}{MOE_{(inhalation)}}}$$

III. CLASSIFICATION OF CANCER POTENTIAL

The HED RfD/Peer Review classified disulfoton as a Group E Chemical-Not Classifiable to Carcinogenicity based on the lack of evidence of carcinogenicity study in mice and rats at dose levels adequate to test for carcinogenicity.

IV. FQPA CONSIDERATIONS

1. Neurotoxicity

Another acute delayed neurotoxicity study (81-7) was submitted and reviewed and is acceptable. The study is negative for organophosphate induced delayed neuropathy (OPIDP). Absolute brain weight was not affected by treatment in the guideline chronic studies in rodents. (The subchronic studies, which were graded unacceptable, were not provided for review.) In the rat study, treatment-related eye lesions were seen (optic nerve degeneration and corneal vascularization) and skeletal muscle atrophy were observed. The optic nerve degeneration was related to orbital sinus bleeding injury, so results were not considered treatment related. These neuropathological findings were not repeated in the 1997 1-year dog study, but cholinesterase levels in the cornea, retina, and ciliary body were depressed with treatment. No treatment related neuropathy was seen in acute or in 90-day neurotoxicity studies in rats. The marginal elevation in lesions seen the optical nerve and thoracic spinal cord at the highest dose tested were not considered to be sufficiently different from control lesions to indicate a treatment-related effect had occurred.

The repeat "acute delayed neurotoxicity study in hens" requested by the HIARC of April 23, 1998 is summarized below.

In an acute delayed neurotoxicity study in hens (MRID# 44996401, 1999), disulfoton was acutely administered orally to 18 LSL laying hens at 40 mg/kg bird in a single dose. Fifteen hens were used as controls. Doses were administered in aqueous 2% Cremophor at 5 ml/kg bird. Five to 18 minutes before administration of the disulfoton, atropine was administered s.c. (0.5 ml/kg of 4% atropine sulfate). Directly prior to the administration of the disulfoton, 0.5 ml/kg of 10% atropine sulfate and 10% 2-PAM chloride was injected s.c. The afternoon of day 0, 0.5 ml/kg of 5% atropine sulfate and 5% 2-PAM chloride was injected s.c. and again the morning and afternoon of day 1. Clinical observations were made at least daily. Forced motor activity tests were conducted by forcing the hens to run around a 12-13 m^2 area and rated for coordination, ataxia, and paresis. NTE studies were conducted at 24 and 48 hours on the spinal cords, sciatic nerves and ½ of the brain in each of 3 hens per group. Cholinesterase activity studies were conducted on the other ½ of the brain from each bird in the NTE study at 24 and 48 hours post treatment. The study was conducted at 1.4 times the LD50 for hens.

No typical signs of organophosphate induced delayed neuropathy was seen during the study or on microscopic examination of the treated birds at termination at 3 weeks. No inhibition was seen in the NTE study at 24 hours or 48 hours. Inhibition was low between 4% and 8% and was not considered to be indicative of OPIDP. Cholinesterase activity in the brain was inhibited 83% and 59% at 24 and 48 hours, respectively.
No hens died, but by day 7 there was a decrease in body weight of over 5%. The hens slowly

recovered and by the end of 3 weeks, body weight of the treatment group and of the controls did not differ.

Severely uncoordinated gait was observed in all treated birds within 5 minutes of being dosed with atropine and before disulfoton treatment. The report authors attributed this abnormal gait to atropine since it lasted only for the duration of the atropine treatment (2 days). However, the report authors also noted reduced motility in 1-3 birds for 0-1 day, which they attributed to disulfoton treatment. Neither statements are completely supportable because the hens were dosed with atropine and disulfoton during most of this period. However, the temporary uncoordinated gait was followed by no microscopic findings in nerve tissue and no other signs, which supports a conclusion of no demonstrated OPIDP in hens dosed with disulfoton.

Microscopic examination of the test birds showed 3 brain (25% - 8% in each region, grade 1) lesions in treated birds and 1 (11%, grade 1) in the same control brain regions. Since these lesions were similar to those found in controls from previous studies, they were considered incidental.

The study supports a conclusion the disulfoton does not cause acute delayed neuropathy (OPIDN) in hens.

The study is acceptable for an acute delayed neurotoxicity study (OPPTS# 870.6100) in hens.

In an acute neurotoxicity study in Sprague-Dawley rats (10/sex/group), 97.8% disulfoton was administered by a single gavage dose of 0.25, 1.5, or 5.0 mg/kg in males and 0.25, 0.75, or 1.5 mg/kg in females. The NOAEL for neurotoxicity and cholinesterase inhibition was 0.25 mg/kg, based on muscle fasciculations in 4/10 females and plasma and RBC cholinesterase inhibition at the LOAELs of 0.75 mg/kg in females and 1.5 mg/kg in males. The incidence and type of clinical, behavioral, and neuromotor signs increased with dose. Females were clearly more sensitive. Neither brain weight nor neuropathology was affected by treatment (MRID 42755801).

In a 90-day subchronic neurotoxicity study, 98.7-99.0% disulfoton was administered to Fischer 344 rats (1 2/sex/group) at dietary levels of 1, 4, or 16 ppm (0.063, 0.270, or 1.08 mg/kg/day in males and 0.071, 0.315, or 1.31 mg/kg/day in females). The systemic NOAEL was 1 ppm (0.063/0.071 mg/kg/day for M/F), based upon clinical signs consistent with cholinesterase inhibition (muscle fasciculations, urine staining, increased food consumption) in females at the LOAEL of 4 ppm (0.270/0.315 mg/kg/day in M/F). At 16 ppm (1.08/1.31 mg/kg/day in M/F), treatment-related findings in both sexes also included increased reactivity, perianal staining, tremors, increased defecation, decreased forelimb grip strength, decreased motor and locomotor activity, decreased body weight gain, and corneal opacities. Cholinesterase inhibition (plasma, erythrocyte, and brain) was observed at all treatment levels (ChE NOAEL$\leq$1 ppm; 0.063/0.071 mg/kg/day for M/F). Clearly females were again shown to be more sensitive. It was noted that clinical signs were persistent throughout this study. There were no treatment-related effects on brain weight. At the high-dose level, neuropathological lesions (nerve fiber degeneration) were observed in the optic nerve, and nerve fiber degeneration was also observed in the thoracic spinal cord. These findings, however, with similar neuropathy in control rats, the marginal increase in these lesions at the highest dose tested were not sufficiently different control lesions to indicate that treatment-related effect had occurred (MRID 42977401).

2. Developmental Toxicity

In a prenatal developmental toxicity study in Sprague-Dawley rats (25/group), 98.2% disulfoton was administered on gestation days 6-15 by gavage in polyethylene glycol 400 at dose levels of 0.1, 0.3, or 1.0 mg/kg/day. Cholinesterase activity was measured in dams (5/group) on gestation day 15. The maternal NOAEL was 0.1 mg/kg/day, and the maternal LOAEL was 0.3 mg/kg/day, based on 41% inhibition of plasma and RBC cholinesterase. There was no other evidence of maternal toxicity at any treatment level. The developmental NOAEL and LOAEL were established at 0.3 and 1.0 mg/kg/day, based on incomplete ossification of the intraparietals and sternebrae (MRID 00129458)

In a prenatal developmental toxicity study conducted in New Zealand white rabbits (15-22/group), 97.3% disulfoton was administered by gavage in corn oil (5 ml/kg) at doses of 0.3, 1.0, or 3.0 (reduced to 2.0, then 1.5) mg/kg/day on gestation days 6-18. The maternal NOAEL was 1.0 mg/kg/day; the maternal LOAEL (1.5 mg/kg/day) was based upon clinical signs of cholinesterase depression (tremors, unsteadiness/ incoordination, and increased respiration, occurring within 4 hours of dosing). In addition, there were a large number of mortalities at the high-dose level. There was no evidence of developmental toxicity (developmental NOAEL $\geq$1.5 mg/kg/day). Neither maternal nor fetal cholinesterase levels were measured (MRID 00147886).

3. Reproductive Toxicity:

In a two-generation reproduction study in Sprague-Dawley rats (25/sex/group), 97.8% disulfoton was administered at dietary concentrations of 1, 3, or 9 ppm (calculated effective doses of 0.81, 2.4, or 76.3 ppm; equivalent to 0.04, 0.12, or 0.36 mg/kg/day mg/kg/day by test material consumption). The parental systemic NOAEL was 3 ppm (0.12 mg/kg/day). The parental systemic LOAEL was 9 ppm (0.36 mg/kg/day), based on decreased females mated and reduced body weight during gestation and lactation in P females. The offspring NOAEL was 1 ppm (0.04 mg/kg/day), and the offspring LOAEL was 3 ppm (0.12 mg/kg/day), based on decreased brain cholinesterase activity in F1a weanling pups and on decreased F2b pup survival. Although adult cholinesterase was not measured, the 2-year chronic study indicates that cholinesterase inhibition was most likely occurring at 3 ppm with a NOAEL of 1 ppm; this was a conclusion of the 4/25/96 RfD PRC meeting (MRID 00157511).

In a another 2-generation reproduction study, disulfoton, technical, 99% a.i.] was administered to 30 Sprague Dawley rats/sex/dose in the diet at dose levels of 0, 0.5, 2.0 or 9.0 ppm (0, 0.025, 0.10 or 0.45 mg/kg/day by std. tables). Dosing was continuous for the P0 and F1 generation. Only one littering/animal/group was conducted. In this second 2-generation reproductive toxicity study with disulfoton, cholinesterase activity was measured in adults during pre-mating (at 8 weeks) and at termination and in pups at postnatal day 4 and day 21 in the 2 generations. The major effects noted were cholinesterase inhibition and dams with no milk. In P0 males, plasma cholinesterase (PCHE) was significantly depressed and dose related pre-mating at 9.0 ppm ($\geq$-34%) and at termination at 2.0 ($\geq$-11%) and 9.0 ppm (-46%). In P0 females, plasma cholinesterase (PCHE) was significantly depressed pre-mating ($\geq$-29%) and at termination ($\geq$-52%) at $\geq$2.0 ppm. In P0 males and females erythrocyte cholinesterase (ECHE) was significantly depressed and dose related at $\geq$2.0 ppm ($\geq$-38% & $\geq$-35% males and $\geq$-46% & $\geq$-80% females) a pre-mating and termination, respectively, but only in females at termination ($\geq$-14%) at $\geq$0.5

ppm. In P0 males and females brain cholinesterase (BCHE) was significantly depressed and dose related at ≥2.0 ppm in males (≥-11%) and ≥-14% in females at ≥0.5 ppm.. PCHE and ECHE depression in F1 males and females followed a similar nominal pattern to that in P0 males and females, except that the statistical significance varied within the F1 between two dose levels; sometimes the dose level showing statistical significance was higher and sometime lower of the two. In F1 males and females, BCHE was significantly depressed and dose related at ≥2.0 ppm in males (≥-14%) and in females (≥-50%). In F1 and F2 male and female pups at day 4 and/or day 21 of lactation, PCHE and ECHE were significantly depressed at 9.0 ppm. Values for PCHE and ECHE, respectively were at day 4 or day 21 in F1 male pups were (-24% & -47%) and for F1 female pups (-31% & -43%). Values for PCHE and ECHE, respectively, were at day 4 or day 21 in F2 male pups were (-46% & -53%) and for F2 female pups (-48% & -51%). In F1 and F2 male and female pups BCHE was significantly depressed at day 4 and day 21 at 9.0 ppm only (day 4 = -14% F1 males and -17% F1 females)(day 21 = -19% F1 males and -23% F1 females)(day 4 = -11% F2 males and -13% F2 females)(day 21 = -35% F2 males and -37% F2 females). Muscle fasciculation (1 P0 female), tremors (15 P0 females, 10 F1 females) and dams (7 F1 dams) with no milk were noted at 9.0 ppm. No treatment related organ weight changes or histopathology were noted in P0 or F1 males or females at any dose level. Clinical observations indicate that dams were not caring for their pups. Observed affects in pups in the 9.0 ppm group included 12 F1 (2 dams) pups cold to the touch and 3 F1 (2 dams) not being cared for and 63 F2 pups (7 dams) with no milk in their stomachs and 93 F2 weak pups (10 dams) from the affected dams. In addition, 1 P0 dam was salivating and gasping and did care for the litter and the litter died at 2.0 ppm. This effect at 2.0 ppm was considered test material related by the summary author of the 6(a)(2) submission (See summary 6(a)(2) report, MRID# 44440801; memorandum from David Anderson to PM 53, dated March 24, 1998, D242573), but ignored in the final report summary. Findings at necropsy were noted in F2 pups at 9.0 ppm that were expected in view of the maternal toxicity at this dose level. The report reasonably considered the pup deaths due to failure of maternal care, because of the weak and cold to the touch pups and failure of the pups to show milk in their stomachs. On careful examination of the report, this reviewer agrees with this conclusion. Thus, under these conditions, the effects in pups were caused by maternal toxicity and not the direct toxicity of disulfoton on pups. Body weight change was lower than control values during gestation in P0 (-9%) and F1 (-15%) females. Body weights were significantly reduced at termination from control values in P0 (-6%) and F1 females (-13%) and in F1 males (-8%). No other significant body weights or changes were noted. The P0 parental LOAELs were 0.5 ppm (0.025 mg/kg/day) based on brain cholinesterase activity depression in P0 females with tremors and muscle fasciculation at 9 ppm in females during gestation and lactation from both generations and with body weight decrements at 9.0 ppm, especially at termination. A NOAEL of 0.5 ppm (0.025 mg/kg/day) was seen in F1 parents. F1 and F2 pup (4 day and 21 day old) cholinesterase activity, including brain cholinesterase activity was depressed only at 9.0 ppm (0.45 mg/kg/day) with 2.0 ppm (0.10 mg/kg/day) being the NOAEL. The F1 pup NOAEL/LOAEL were 2.0/9.0 ppm (0.10/0.45 mg/kg/day) based on treatment related pup deaths and pup weight decrements at 9.0 ppm, probably from inadequate maternal care (MRID# 44440801).

4. Additional Information from the Literature

This summary is provided to develop a comprehensive picture of disulfoton toxicity. The data

have not been reviewed in depth, and no statement is made regarding the accuracy or quality of the data or reports.

In a 1988 study by McDonald et al., disulfoton was administered by daily i.p. injection at 2 mg/kg/day to male Long-Evans rats for 14 days. In treated rats, muscarinic receptor binding was decreased and spacial memory was decreased in a T-maze alternation task.

5. Determination of Suseptibility

There is no indication of increased susceptibility of fetuses, infants or children over adults to disulfoton from developmental toxicity studies in rats and rabbits or from two 2-generation studies on reproduction. In these studies, toxicity to the fetus or pups occurred only at higher dose levels than to the adults (dams or parents).

6. Recommendation for Developmental Neurotoxicity Study

A developmental neurotoxicity study with disulfoton is required by the Data Call-In Notice (September 10, 1999) for select organophosphates. Based on the following weight-of-the-evidence considerations, the HIARC concluded that this study would not have been otherwise triggered.

- Developmental toxicity studies showed no increased susceptibility in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.

- The two-generation reproduction toxicity studies in rats showed no increased susceptibility in pups when compared to adults. In addition, the pup deaths at the highest dose level in the second study on reproduction were due to a failure of maternal care and not due to direct toxicity from disulfoton.

- There was no evidence of abnormalities in the development of fetal nervous system in the pre/post natal studies.

- There was not evidence of organophosphate induced delayed neuropathy (OPIDN) in the acute delayed neurotoxicity study in the hen.

- No clinical signs of indicative of neurotoxicity or neuropathy was seen in the acute neurotoxicity study in rats.

- In the subchronic neurotoxicity study in rats, at the high dose, neuropathological lesions (nerve fiber degeneration) were observed in the optic nerve, optic nerve, and nerve fiber degeneration was also observed in the thoracic spinal cord. These findings, however, were not judged to be sufficient evidence of treatment-related neuropathology, since there similar lesions occurred in control rats and there was an insufficient difference in the high dose animals show that an effect had occurred.

7. Determination of the FQPA Safety Factor:

The final recommendation on the FQPA Safety Factor will be made by the FQPA Safety Committee.

V. DATA GAPS

There are no data gaps. However, disulfoton was included in the Data Call-In Notice (September 10, 1999) for the requirement of a developmental neurotoxicity study.

VI. HAZARD CHARACTERIZATION

Cholinesterase inhibition (plasma, erythrocyte and/or brain) is seen at the lowest dose levels tested in rats, mice, rabbits and dogs. All the endpoints are based on good dose related responses in cholinesterase inhibition. Many of the studies show clinical signs at higher dose levels. Females appear to be more sensitive to cholinesterase inhibition in most studies.

The organophosphates have a common mode of action in that they decrease erythrocyte and/or brain cholinesterase in animals and humans. Plasma cholinesterase inhibition is a surrogate for possible muscle and brain cholinesterase inhibition. Neuropathy may result from higher exposures to these inhibitors. The rabbit 21-day dermal study did not show as consistent cholinesterase inhibition with time as other studies showed. The results were somewhat dependent on whether concurrent controls were used or the values for the individual animals at the beginning of the study were used for comparison.

Cholinesterase inhibition occurred at the LOAEL in rats, mice, rabbits and dogs. Therefore the effects are very uniform across species. The female of the species appears to be more sensitive than the male and the cholinesterase inhibition occurs at slightly different dose levels across the species. The cholinesterase inhibition appears to be slightly greater in the female than the male in most studies. There is insufficient studies with common dosage regimens to determine the most sensitive species except that the rat is more sensitive than the mouse in oncogenicity studies.

The dose level causing plasma cholinesterase inhibition was 1/3 that causing death in the rabbit dams in the developmental toxicity study. The LOAEL causing brain cholinesterase inhibition in parents was 1/45 of the dose level resulting in offspring mortality in the second 2-generation reproduction study. In the acute neurotoxicity rat study reduced motor function occurred at the LOAEL for plasma cholinesterase inhibition (ChEI), red blood cell ChEI and brain ChEI. In the 90-day neurotoxicity study plasma ChEI, RBC ChEI and brain ChEI occurred at about 1/4 (0.063/0.27) the dose level resulting clinical signs.

Adequate developmental toxicity and reproductive toxicity studies show adult toxicity occurs at lower dose levels than toxicity to the fetus or offspring. There is no evidence to support increased susceptibility following pre natal exposure to rat or rabbit fetuses or pre/post natal exposure to rats. In these studies, toxicity to the fetus or pups occurred only at higher dose levels than to the adults (dams or parents). Thus, there is no evidence of increased susceptibility to the fetus or to offspring.

The following literature summary is provided to develop a comprehensive picture of disulfoton toxicity. The data have not been reviewed in depth, and no statement is made regarding the accuracy or quality of

the data or reports. n a 1988 study by McDonald et al., disulfoton was administered by daily i.p. injection at 2 mg/kg/day to male Long-Evans rats for 14 days. In treated rats, muscarinic receptor binding was decreased and spacial memory was decreased in a T-maze alternation task. These effects occurred in the presence of -75% brain ChEI therefore the effects may not be relevant at the NOAEL for brain ChEI.

VII. ACUTE TOXICITY ENDPOINTS:

Acute Toxicity of disulfoton, technical

Guideline No.	Study Type	MRID #(S).	Results	Toxicity Category
81-1	Acute Oral	Acc# 072293	LD_{50} = M: 6.2 mg/kg; F:1.9 mg/kg	I
81-2	Acute Dermal	Acc# 07793	LD_{50} = M: 15.9 mg/kg; F: 3.6 mg/kg	I
81-3	Acute Inhalation	Acc# 258569	LC_{50} = M: 0.06 mg/L; F: 0.89 mg/L	I
81-4	Primary Eye Irritation	None	Data requirement waived.	N/A
81-5	Primary Skin Irritation	None	Data requirement waived.	N/A
81-6	Dermal Sensitization	None	Data requirement waived.	N/A
81-7	Acute Delayed Neurotoxicity	00129384	Equivocal	
81-8	Acute Neurotoxicity	42755801	Reversible neurotoxic signs consistent with the cholinesterase inhibition 1.5 mg/kg in females and 5.0 mg/kg in males	N/A

VIII. SUMMARY OF TOXICOLOGY ENDPOINTS

The doses and toxicological endpoints selected for various exposure scenarios are summarized in the table below.

Exposure scenario	Dose (mg/kg/day)	Endpoint	Study
Acute Dietary	NOAEL=0.25	Cholinesterase/clinical signs	Acute neurotox/rat
Acute dietary RfD = 0.0025 mg/kg/day			
Chronic dietary	NOAEL=0.013	Cholinesterase inhibition	Chronic/Dog
Chronic dietary RfD = 0.00013 mg/kg/day			
Short-term (Dermal)	Dermal NOAEL=0.4	Cholinesterase	21-day dermal/rabbit
Correction for dermal absorption unnecessary			
Intermediate-term (Dermal)	Oral NOAEL=0.03	Cholinesterase inhibition	6-months chronic/rat
Correction for oral to dermal exposure necessary (36% dermal absorption factor)			
Long-term (Dermal)	Oral NOAEL=0.013	Cholinesterase inhibition	Chronic/dog
Correction for oral to dermal exposure necessary (36% dermal absorption factor)			
Inhalation (Any time period)	NOAEL=0.00016 mg/L	Cholinesterase inhibition	90-day inhalation/rat

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